



Croatian Society of Toxicology

Factors affecting toxicity

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Modification of the toxicity of chemicals is important because of

- possible increased or decreased toxicity of toxin or drug given to animal or humans
- possible erroneous interpretation of own results or evaluation of studies on experimental results in Hazard Identification which is part of Risk Assessment

Toxic effects of chemicals are in relation with

- Bioavailability which depends on absorption, distribution, and excretion of toxic chemicals
- Metabolism
- Sensitivity of receptors on target organs

Toxic effects of chemicals can be modified by

- Chemical factors
- Factors associated with treatment
- Biological factors
- Environmental factors

Chemical factors

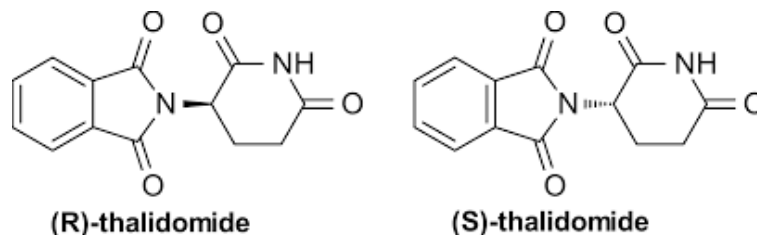
- Lipophilicity
- Structure
- Ionization
- Chilarity

Chemical factors

Chirality

Chirality is property of molecules with one central atom (usually carbon) with four different atoms or groups attached on it. Such nonsuperimposable mirror image structures are called enantiomers or optical isomers. Optical isomers have different ability to rotate a beam of polarized light: dextrorotatory (+) and levorotatory (-) enantiomer. Mixtures of dextrorotatory and levorotatory enantiomers are called racemic mixtures.

Thalidomide



Very popular sedative and mild hypnotic, used in pregnancy to cure anxiety, insomnia, gastritis and tension.

There are two optical isomers of Thalidomide which is racemic mixture of (-)(S)-thalidomide (teratogen) and (+)(R)-thalidomide (effective sedative).

Chemical factors

Chilarity

Side effect of (-)(S)-thalidomide is the inhibition of the growth of new blood vessel.

Inhibition of the growth of new blood vessels is detrimental for fetus because new blood vessels provide „road map” for the growth of limbs and organs.

In early 60's last century Thalidomide caused phocomelia (malformation of limbs) and other malformations (brain, heart, liver, kidney, eyes and ears). Out of 10,000 babies survived 50 %.

Nowadays Thalidomide is used because of antiangiogenic properties in treatment of multiple myeloma and complications of leprosy (erythema nodosum).

Factors associated with treatment

Dose

Volume of solvent

Type of exposure

- acute – usually results with high concentration at the target site which may cause acute or delayed effects

Route of exposure

- intravenous and intraperitoneal applications result in higher toxicity because the toxin enters circulation avoiding liver which is the most important organ for detoxication of xenobiotics. There is no „first pass effect” as after oral treatment.

Factors associated with treatment

Animal husbandry

- Animal husbandry and a variety of social factors can modify toxicity of chemicals.
- The handling of animals, the housing (single or in groups), the type of the cage and the bedding materials are important factors.
- Animals require quite and friendly atmosphere.

Biological factors affecting toxic response

- Species and strain of the animal
- Sex
- Age
- Anatomic and physiological conditions
- Diseases
- Diet and nutritional status
- Chemicals in the diet, air or water
- Human variability

Biological factors affecting toxic response

Species and strain differences

Species differences in toxicity of a compound are related to differences in metabolism and disposition of the compound.

Disposition of the compound

- Absorption of the compound through skin shows considerable species variation
- Oral absorption depends upon
 - pH of digestive tract (herbivores vs. carnivores)
 - quantity of food in the gastrointestinal tract. Food decreases drug absorption because of binding of toxic compounds to food proteins.
 - fasting causes higher production of ketones which induce CYP 2E1 which increases metabolism of low MW xenobiotics (acetaminophen, ethanol, N-nitrosomethylamine)
- Absorption by inhalation depends upon breathing rate (smaller animals have much higher ventilation rates than larger animals)

Biological factors affecting toxic response

Species and strain differences

Disposition of the compound

Distribution

The plasma protein concentration is a species-dependent variable, also types of proteins vary between species.

Excretion

Rate of urine excretion varies considerably between species (in rat 10 times higher than that of humans).

The molecular weight cut-off for biliary excretion shows considerable species variations (threshold in rat is 325, in guinea pig 400, in rabbits 475, in humans is 500-700).

Biliary excretion depends on pH of digestive tract segments and composition of micro flora in segments.

Biological factors affecting toxic response

Species and strain differences

Metabolism

Small animals metabolize compounds at a faster rate than large animals per unit of body weight.

Differences are mostly quantitative but there are also some qualitative differences.

Biological factors affecting toxic response

Species and strain differences

Metabolism

Phase I. reactions

Most common differences are in rate at which particular compound is oxidized but there are also some pathway differences.

Ethylene glycol toxicity

- production of oxalic acid is in order:
- cat > rat > rabbit
- toxicity of ethylene glycol is in same order
- oxalic acid precipitates in the kidney tubules forming casts and kidney damage

Biological factors affecting toxic response

Species and strain differences

Metabolism

Phase II. reactions

Glucuronide conjugation is an important route of metabolism in mammals, birds, reptiles and amphibians, but not in fish.

Sulphate conjugation – found in most mammals, birds, reptiles and amphibians, but not in fish.

Amino acid conjugation is favored in herbivores, glucuronide conjugation is favored in carnivores and omnivores utilize both.

Biological factors affecting toxic response

Species of experimental animals

Target organ is the primary or most sensitive organ affected after exposure. The same chemical entering the body by different routes of exposure dose, dose rate, sex and species may affect different target organs.

The same target organ in different species is frequent (aflatoxin B1, chlorophorm, ochratoxin A, mercury).

Aflatoxin B1 is hepatotoxic in all animal species. Acute aflatoxin B1 LD₅₀ (mg /kg, p.o.) in various animal species.

rabbit	0,3
cat	0,55
dog	0,55-1,0
pig	0,6
trout	0,8
guinea pig	1,0-1,4
rat (1 day old)	1,0
rat (adult)	6,0-7,0
hen	6,3
macaque monkey	7,8
mouse	9,0
hamster	10,2

Biological factors affecting toxic response

Species of experimental animals

Different target organs in different species:

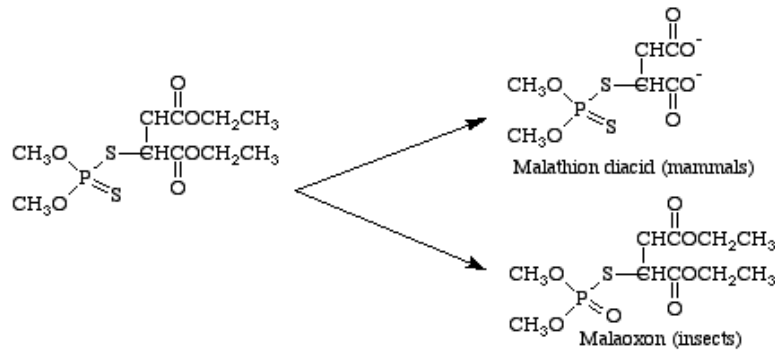
Mycotoxin Fumonisin B1 is compound that in various species has different target organs of toxicity:

- brain – in horses – leukoencephalomalacia
- lungs – in pigs – pulmonary edema
- kidney – rat and mouse – more nephrotoxic in rat than in mouse

Some organophosphate compounds may cause syndrome called Organophosphate induced delayed neuropathy (OPIDP) that develops 2-3 weeks after severe acute intoxication. OPIDP could be studied only in poultry (hen, turkey, quails) but other experimental animals are resistant.

The use of species differences as „Selective toxicity”

- Insects oxidize malathion in highly toxic malaoxon, while mammals hydrolyze this OP compound and for them it is not toxic.



- Rodents do not have reflex of vomiting – anticoagulant warfarin is used as a rodenticide that can't be spit out by rodents.
- Differences in response of mammals and bacteria is used in the antibiotic treatment of bacterial infections.

Biological factors affecting toxic response

Individual variations within the same stock

Toxicological studies are performed mostly on outbred stocks which are „genetically undefined“. Even when the study is performed on the same stock there are always the individual differences in toxic response.

The term „stock“ is used for outbred animals and „strain“ for inbred animals.

Inbred animals are matched in at least 20 generations among brothers and sisters with all offspring being derived from a single pair of animals. They are regularly used in genetic studies. Properties of inbred animals are fixed, with all individuals being homozygous. The only way an inbred strain can change is a result of new mutations.

Biological factors affecting toxic response

Sex-related variabilities

Usually male and female animals have similar toxic response to the same compound. When differences exist they are mostly quantitative and not qualitative.

Male rats metabolize xenobiotics more rapidly than females.

Humans are similar in that aspect to rat because men metabolize xenobiotics faster than woman.

Female mice metabolize compounds more rapidly than male mice.

When toxicity is sex-related, toxic response of males becomes similar to toxicity in females after castration. This can be reversed if testosterone therapy is applied in castrated animals.

Biological factors affecting toxic response

Sex- related variabilities

Barbiturates induce longer sleep in females than in males that have higher activity of liver microsomal enzymes for hydroxylation of hexobarbital.

Chloroform is one of the first anaesthetic which is abandoned because it may cause lethal arrhythmias or respiratory arrest. It is also hepatotoxic with more severe liver damage in male than in female mice. In males rats testosterone induces liver microsomal enzymes thus causing higher metabolism of chloroform and production of phosgen which is hepatotoxic.

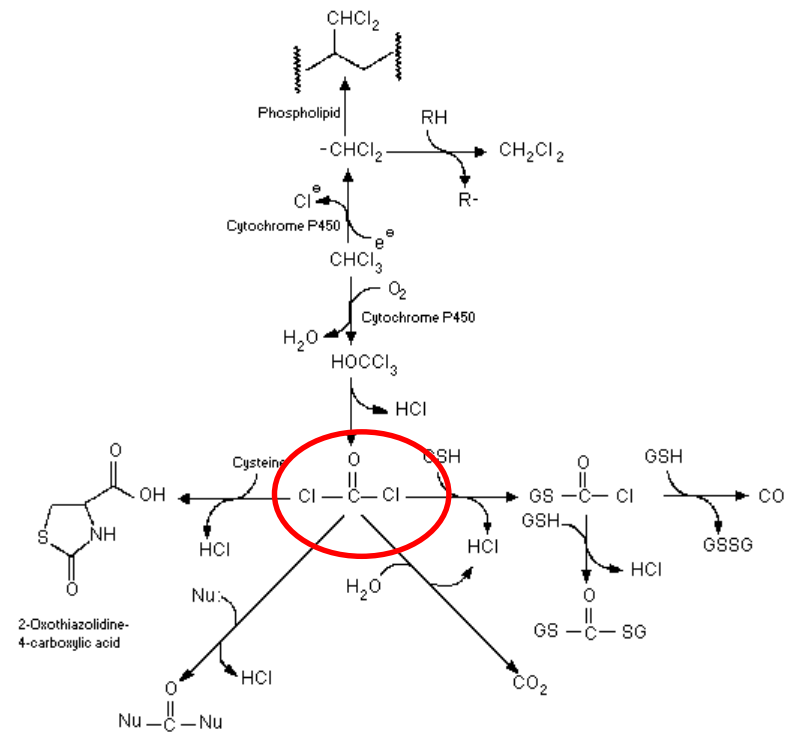


Fig. 1. Biotransformation of chloroform

Biological factors affecting toxic response

Age-related variabilities

Various toxicants are more toxic in young animals than in adults because of:

- undeveloped mechanisms of detoxication
 - Aflatoxin B1 is 7 times more toxic in 1 day old rat than in adults
- undeveloped blood-brain barrier
- higher absorption of certain compounds
 - lead is absorbed 4-5 times more by the young than by adults

Exception:

Young animals and children have fewer symptoms and recover completely from OPIDP after severe intoxication with some organophosphates. This is due to the very quick de novo synthesis of the target enzyme in peripheral nerves.

Biological factors affecting toxic response

Age-related variabilities

Various toxicants are also more toxic in old animals and humans because of:

- reduced detoxication
- impaired renal excretion
- decreased blood flow
- lower level of total plasma proteins

Distribution of toxicants and drugs may be altered because of increased body fat and decreased body water.

Biological factors affecting toxic response

Diseases

- Acute and chronic liver injury such as hepatitis, cirrhosis, toxic hepatitis, porphyria, and liver tumors will affect the biotransformation.
- Renal disease can cause disturbances in excretion of a chemical thus increasing its toxicity.
- Severe heart diseases can increase toxicity of chemical by impairing the renal and hepatic circulation.
- Respiratory tract disorders such as asthma render the subjects more susceptible to air pollutants.

Biological factors affecting toxic response

Diet and nutritional status

- A deficiency of essential fatty acids and proteins generally depress cytochromes activity. This could modify the toxicity of chemicals.
- In humans toxicity of chemicals may be affected by poor nutrition. Such diet may decrease biotransformation capacity.
 - Aflatoxin B1 was considered to be the causative agent of Kwashiorkor disease in Africa because it was found frequently in biological materials at postmortem. Reason for this finding was the decreased metabolism of this mycotoxin.

Biological factors affecting toxic response

Chemicals in the diet, air or water

Humans may be under medication with several drugs while exposed to an industrial chemical.

The toxicity of a chemical in an organism may be increased or decreased by a simultaneous or consecutive exposure to another chemical in food.

- grapefruit juice increases the bioavailability of immunosuppressive drug Cyclosporin A by inactivating the CYP 3A4 in liver and small bowel enterocytes. Cyclosporin A is used for immunosuppression in renal allograft recipients.

- polyphenol epigallocatechin gallate from green tea prevent tumor death induced by antitumor drug Bortezomib (proteasome inhibitor) used in treatment of multiple myeloma and mantle cell lymphoma). Green tea decreases the effect of Bortezomib.

Cigarette smoking and alcohol intake are also known to affect drug metabolism and pharmacological response.

Biological factors affecting toxic response

Human variability

Toxic response in humans may be very different because of genetic variability of humans.

Glucose-6-phosphate dehydrogenase deficiency (G6PD) is X-linked genetic condition that predispose to hemolytic anemia. Called also favism, it is common in persons from Mediterranean and African origin. G6PD-affected males are immune to malaria.

Manifestations of G6PD are:

- - prolonged neonatal jaundice with possible kernicterus
- - hemolytic crises caused by illness (infections), drugs (antimalarial drugs primaquine, pamaquine, chloroquine), foods (broad beans, *Vicia faba*)
- - severe crisis can cause acute kidney failure

Biological factors affecting toxic response

Human variability

- Most frequent human enzyme defect is present in about 400 million people.
- In 2013 favism caused 3400 deaths.
- Problem of malaria is still very serious.
- In 2015 the Nobel prize winner was dr. Youyou Tu from China because she found a new antimalaric drug.



Thank you

for your attention!